COMPARISONS OF THE RATE OF GAS-PRODUCTION BY CERTAIN BACTERIA IN RAW AND IN PASTEURIZED MILK *

Paul W. Allen

From the University of Illinois, Urbana, Ill.

In most of the work concerning the activity of bacteria in raw milk as contrasted with that in heated milk it has been found that there is a real suppression of the rate of multiplication in the raw during the first few hours after milking. However, the explanation of the cause of this phenomenon has differed widely. The method of determination of suppression of bacteria has generally been the plating of the sample at regular intervals of time, using the plate count as the basis for arriving at conclusions. A number of the ablest bacteriologists since 1890 have contributed to this subject.

The earlier workers believed that milk like blood contains protective bodies. Because of this view a milk diet was often advised in some diseases. But it was soon discovered that this much talked-of protective property is a minor factor in dealing with contamination of market milk. Later it was held that raw milk does have an inhibitive influence which modifies its germ content considerably. Some state that pasteurization (60 C. for 30 minutes) partly removes this inhibitive influence, and they hold that this fact demands consideration in the handling of pasteurized market milk.

OBJECT OF EXPERIMENT

In the study of bacteria in nature, we are often impressed with the fact that some of the most extensive and most powerful forces are those which suppress rather than annihilate. This seems to be true concerning bacterial action. In the case of nonpathogenic bacteria we are, as a general rule, more concerned with their suppression than with their extermination. For instance, it may be true in the case of fresh milk that while its germicidal action may be weak or more likely actually absent, its suppressive influence on bacteria may be an important consideration in the use of fresh milk as food.

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TABLE 1

RESULTS OF MILK FERMENTATIONS

Fermentation Series Number	Date	Milk Used, C.c.	Number Germs per c.c. in Milk Before Inoculation	Kind of Organism Used for Inoculation	Number of Organisms Used for Inoculation
6	4/16	500	312	B. coli	500,000,000
7	4/16	350	627	B. coli	11,470,000,000
8	4/16	500	316	B. coli	840,000,000
14	11/16	22	16,100	B. aerogenes	20,000,000
15	12/16	22	18,260	B. aerogenes	20,000,000
16	12/16	22	1,140	B. aerogenes	20,000,000
17	12/16	22	1,720	B. aerogenes	20,000,000
18	12/16	22	5,565	B. aerogenes	20,000,000
19	12/16	22	3,500	B. aerogenes	20,000,000
20	12/16	22	6,016	B. aerogenes	20,000,000
21	12/16	22	3,515	B. aerogenes	20,000,000
22	12/16	22	9,150	B. aerogenes	20,000,000

For the purpose of determining the presence of or defining the limits of such an inhibiting influence in raw milk and its reduction or loss in the same when heated, the use of parallel samples for comparisons lends itself exceptionally well. However, this method of procedure has been used very little. Most workers have turned their efforts in this direction with the object in view of either proving or disproving the presence of a germicidal action.

In using aliquot samples of raw and pasteurized milk inoculated with equal amounts of the same pure culture of gas-forming bacteria and subjecting them to the same conditions, we have a test that enables us to detect certain kinds of changes which cannot be equaled for delicacy by chemical analysis. We are employing here biologic measurements, and differences not chemically appreciable become important when interpreted in terms of growth and function.

In this paper it is not the intention to try to explain in any case differences between the physiologic activities of bacteria in raw and in heated milk by naming a specific cause or causes, but simply to determine the presence of differences with the idea of obtaining thereby some rough indication of the kind of factors dealt with.

Among the physiologic activities of micro-organisms some of the more easily measured are multiplication, gas-production, acid-production, alkali-production, and peptonization. Of these, some work has been done toward the measurement of differences in rate of multiplication of certain bacteria in raw and heated milk. The results given here take up mainly measurements of gas-production by bacteria in raw and in heated milk.

TABLE 1—*Continued*

RESULTS OF MILK FERMENTATIONS

Temperature of Incubation, C.	Total Hours of Fermentation	Total Gas Formed in Triplicate Tubes in Raw Milk			Total Gas Formed in Triplicate Tubes in Pasteurized Milk		
		1	2	3	1	2	3
37	16	11.4	8.5	10.3	22.4	22.7	20.7
37	17	19.7	19.9	22.4	25.8	26.0	35.1
37	19	17.5	15.8	16.2	24.1	26.7	22.3
37	72	5.5	6.9	7.1	11.5	10.0	9.6
37	72	6.5	7.4	7.0	9.6	8.5	8.2
30	72	7.2	6.8	5.5	7.4	7.0	8.6
30	72	5.0	5.6	6.1	6.3	5.2	7.2
30	72	6.2	7.9	6.8	9.6	8.2	8.1
30	72	3.8	4.7	5.3	7.3	7.4	7.2
30	72	2.8	4.4	3.5	3.8	5.4	6.9
30	72	7.0	7.4	5.6	12.8	10.5	11.6
30	72	4.8	6.5	6.3	7.8	11.7	8.0

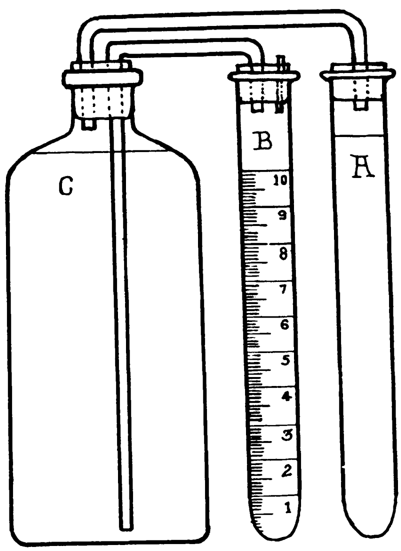

Fig. 1.—Apparatus for fermentation, 3 of which are used in obtaining triplicate results on a single fermentation. A, fermenting liquid; B, graduated test tube for measuring displaced water; C, overflow bottle containing water.

METHOD

In the comparisons of physiologic activity of bacteria in milk all factors were the same with the exception that part of the milk was raw and part was pasteurized (60 C. for 30 minutes).

(1) The milk used was divided into 2 parts (a) pasteurized, and (b) raw.

(2) Triplicate samples of (a) and (b) were inoculated with an equal number of gas-producing organisms using the water suspension method in inoculating quantitatively. Great pains were taken to bring all samples to the same temperature before inoculation.

(3) All samples were incubated at the same temperature.

(4) The amount of gas was determined at the end of each hour.

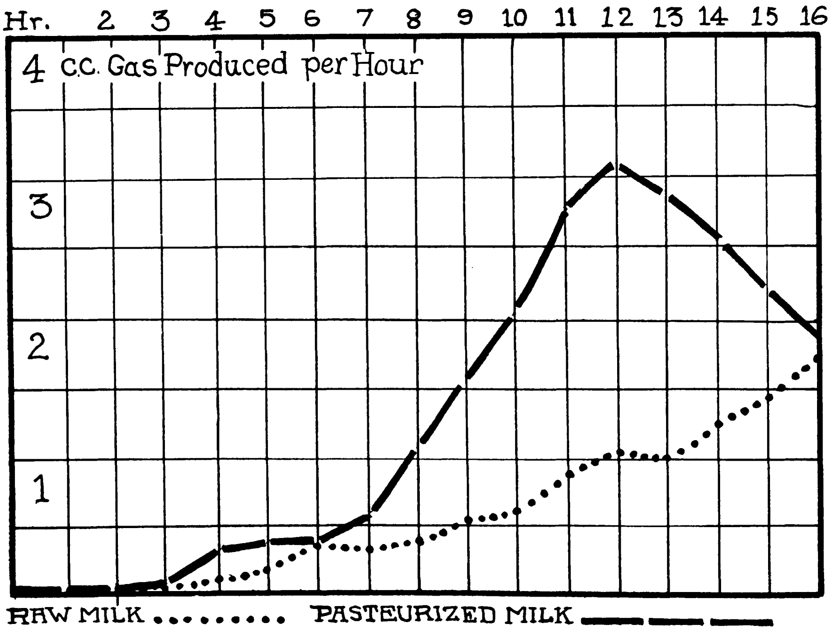

Fig. 2.—Fermentation 8.

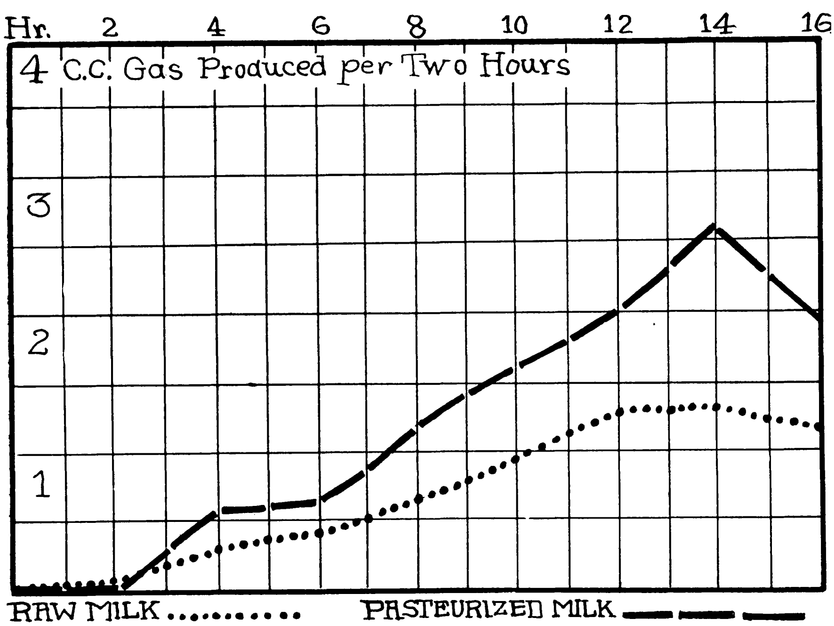

Fig. 3.—Fermentation 21.

TABLE 2

AVERAGE GAS PRODUCED PER HOUR IN TRIPLICATE TUBES OF MILK IN FERMENTATION SERIES 6, 7, AND 8

Hour	Fermentation					
	Series 6		Series 7		Series 8	
	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.
1	0	0	0	0	0	0
2	0	.06	0	.03	0	0
3	.03	.16	.06	.4	0	.16
4	.16	.3	.1	.23	.03	.2
5	.23	.3	.16	.76	.13	.36
6	.4	.4	.33	.9	.2	.5
7	.3	.56	.23	.166	.16	.83
8	.4	1.16	.46	2.33	.2	.76
9	.53	1.59	1.3	2.6	.4	1.0
10	.63	2.06	1.8	3.26	.56	1.06
11	.86	2.73	2.0	3.6	.83	1.26
12	1.1	3.16	2.23	3.4	1.1	1.43
13	1.0	2.83	2.46	2.83	1.33	1.73
14	1.26	2.63	2.43	2.4	1.5	2.1
15	1.4	2.23	2.33	1.86	1.6	2.26
16	1.73	1.73	2.43	1.36	1.76	2.7
17	.....	.....	2.3	1.2	2.06	2.76
18	.....	.....	.....	.....	2.26	2.6
19	.....	.....	.....	.....	2.33	2.6
	10.06	21.93	20.66	28.96	16.50	24.36

TABLE 3

AVERAGE GAS PRODUCED PER HOUR IN TRIPLICATE TUBES OF MILK IN FERMENTATION SERIES 18, 19, 20, 21, AND 22

Hours	Fermentation Series 18		Fermentation Series 19		Fermentation Series 20		Fermentation Series 21		Fermentation Series 22	
	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.	Raw, C.c.	Pasteurized, C.c.
2	.26	.13	0	0	0	0	.13	.1	0	0
4	.4	.66	.3	.3	0	.03	.33	.56	.2	.16
6	.36	.73	.46	.56	.13	.33	.43	.66	.53	.56
8	.66	1.03	.66	.86	.26	.53	.7	1.2	.83	1.06
10	.86	1.1	.86	1.1	.53	.93	.93	1.63	.86	1.86
12	1.26	1.26	.83	1.33	.7	1.23	1.23	2.0	.83	2.06
14	1.43	1.5	.96	1.53	.66	1.56	1.33	2.6	1.2	1.6
16	1.3	1.6	.46	1.2	.8	.86	1.2	1.9	.83	1.63
24	.4	.6	0	.36	0	0	.36	.96	.3	0
48	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0
	6.96	8.63	4.6	7.3	3.56	6.03	6.66	11.63	5.86	9.16

TABLE 4

A TYPICAL SERIES WITH THE COLON BACILLUS; MILK FERMENTATION SERIES 8

Hours	Gas Produced per Hour in Triplicate Tubes of Raw Milk, C.c.				Gas Produced per Hour in Triplicate Tubes of Pasteurized Milk, C.c.			
	1	2	3	Average	1	2	3	Average
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	.2	.1	.2	.16
4	0	0	.1	.03	.2	.2	.2	.2
5	.2	.1	.1	.13	.4	.5	.2	.36
6	.2	.2	.2	.2	.4	.6	.5	.5
7	.1	.2	.2	.16	.7	1.2	.6	.83
8	.3	.1	.2	.2	.7	1.1	.5	.76
9	.5	.2	.5	.4	.7	1.2	1.1	1.0
10	.6	.5	.6	.56	.9	1.2	1.1	1.06
11	.9	.7	.9	.83	1.2	1.4	1.2	1.26
12	1.0	1.0	1.3	1.1	1.5	1.6	1.2	1.43
13	1.3	1.2	1.5	1.33	1.8	1.9	1.5	1.73
14	1.5	1.6	1.4	1.5	2.4	2.2	1.7	2.1
15	1.9	1.3	1.6	1.6	2.4	2.6	1.8	2.26
16	2.0	1.6	1.7	1.76	2.9	3.0	2.2	2.7
17	2.2	2.1	1.9	2.06	2.7	3.0	2.6	2.76
18	2.5	2.4	1.9	2.26	2.5	2.6	2.7	2.6
19	2.3	2.6	2.1	2.33	2.5	2.3	3.0	2.6
Total at end of 19 hours	17.5	15.8	16.2	16.5	24.1	26.7	22.3	24.36

TABLE 5

A TYPICAL SERIES WITH B. AEROGENES; MILK FERMENTATION SERIES 21

Hours	Gas Produced During 2-Hour Intervals in Triplicate Tubes of Raw Milk, C.c.				Gas Produced During 2-Hour Intervals in Triplicate Tubes of Pasteurized Milk, C.c.			
	1	2	3	Average	1	2	3	Average
0	0	0	0	0	0	0	0	0
2	0	.1	.3	.13	0	.3	0	.1
4	.4	.3	.3	.33	.5	.5	.7	.56
6	.4	.6	.3	.43	.9	.3	.8	.66
8	.7	.9	.5	.7	1.4	.7	1.5	1.2
10	1.0	1.2	.6	.93	1.5	1.6	1.8	1.63
12	1.3	1.4	1.0	1.23	1.8	1.9	2.3	2.0
14	1.1	1.4	1.5	1.33	2.6	2.5	2.7	2.6
16	1.6	.9	1.1	1.2	2.5	2.2	1.0	1.9
24	.5	.6	0	.36	1.6	.5	.8	.96
48	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0
Total at end of 72 hours	7.0	7.4	5.6	6.66	12.8	10.5	11.6	11.63

NOTES CONCERNING FERMENTATION

Triplicate Smith fermentation tube checks were made with the milk used in each fermentation series here reported and resulted in the absence of gas-formation in each case.

The milk used in Series 6, 7, and 8 was obtained in sterile bottles at the barn at milking time while in the outer fermentation series the milk used was obtained from the cans immediately after milking.

The curves given in Figs. 2 and 3 of the rate of gas-production cannot be compared with curves showing rate of increase of total numbers of organisms, as in the former the curves represent at any given time the gas produced per unit of time, while rate of multiplication curves generally represents the total number of organisms accumulated at any given time. If the curves had been drawn on the basis of the total amount of gas accumulated at the end of intervals of time much greater contrast in the courses of the curves would have appeared.

CONCLUSIONS

Pasteurization causes milk to become more favorable to the attack of the gas-forming colon bacillus and B. aerogenes. These results seem to reinforce the impression long held by many milk men that pure raw milk has a power of resisting changes which the same milk does not possess when pasteurized.

Between raw and pasteurized milk there may be important differences, although chemical analyses may show no appreciable differences.

In view of the fact that milk has its value strictly because of its relation to growth, in studying raw and heated milks, due consideration should be given to delicate biologic tests which utilize growth as the means of producing comparative data.

It should be more generally recognized that pasteurized milk instead of receiving less care than raw milk should receive greater care because of its lessened resistance to many detrimental changes which the appearance of the milk does not indicate. This is especially significant in that, in general, pasteurization has lengthened the period between production and consumption.